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PRINCIPAL INVESTIGATOR: Daniel Vigneron, Ph.D

CONTRACTING ORGANIZATION: University of California, San Francisco
San Francisco, CA 94143-0962

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Daniel B. Vigneron, PhD

E-Mail: dan.vigneron@ucsf.edu

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14. ABSTRACT
The goal of this project is to develop a new MRI approach to characterize aggressive cancers based on their genetic/proteomic and perfusion abnormalities and apply this method in preclinical models to obtain the required preliminary data for FDA approval for future clinical trials. The initiation and first year of this project have gone extremely well accomplishing the technical milestones described in the original approved Statement of Work (SOW). No changes in the SOW were needed (or done). In this first year, the technical developments were accomplished and transgenic mouse model studies were initiated. Following the technical development phase, we have initiated the preclinical studies in the transgenic model of prostate cancer studying both Early Stage and Late Stage cancers. Over the past 6 months we have tested and optimized the techniques *in vivo* and to date have studied a total of nine mice. The initial results demonstrate feasibility and support the hypothesis that this new approach could distinguish aggressive from indolent prostate cancer. Now that we have the techniques working well, we are confident that we can complete the proposed number of studies by the end of the project.

15. SUBJECT TERMS
Magnetic Resonance Imaging, Prostate Cancer, Aggressiveness, Cancer Characterization, Metabolism, Perfusion, Non-Invasive, Hyperpolarized Carbon-13, Mutation Detection, Quantitative Imaging, Pre-clinical.

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1. INTRODUCTION:

A pressing need facing the clinical management of prostate cancer is an accurate method for distinguishing aggressive prostate cancer from indolent disease (a PCRP Overarching Challenge). The American Cancer Society estimates that 241,740 men will be diagnosed with prostate cancer in the United States in 2012, which is a higher incidence figure than any other non-cutaneous human malignancy [1]. There is currently no cure for metastatic prostate cancer and an estimated 28,710 men will die of the disease in the United States in 2012, a figure surpassed only by lung cancer [1]. The decision on how to manage prostate cancer poses a great dilemma for patients and their physicians because prostate cancers demonstrate a tremendous range in biologic diversity and are treated with a broad spectrum of approaches from "active surveillance" to aggressive surgical, radiation and other focal therapies [2]. Such therapies have tradeoffs since treatment is frequently associated with changes in health-related quality of life [3] [4]. Moreover, many prostate tumors follow such an indolent course that they might never threaten the duration or quality of lives of affected men if left untreated. Unfortunately, differentiation of aggressive prostate cancers from indolent disease cannot be confidently predicted in individual patients using current prognostic markers [5] [6]. This project is developing a new MRI approach to characterize aggressive cancers based on their genetic/proteomic and perfusion abnormalities and apply this method in preclinical models to obtain the required preliminary data for FDA approval for future clinical trials. Hyperpolarized (HP) carbon-13 MRI is a powerful new molecular imaging method which uses specialized instrumentation to provide signal enhancements of over 5-orders of magnitude for carbon-13 enriched, safe, endogenous, non-radioactive compounds as described in a recent NIH-supported "White Paper" [7]. The first human Phase 1 clinical trial has demonstrated the safety and feasibility of HP ^{13}C-pyruvate MRI approach in prostate cancer patients [8]. This project aims, for the first time, to develop and test a new dual-agent HP MRI approach for simultaneous metabolic + perfusion imaging using HP-pyruvate and also HP urea to detect altered vascular perfusion in aggressive prostate cancers in a preclinical transgenic model. HP urea is not metabolized and can provide an excellent internal-standard measure of perfusion to detect altered blood flow parameters in cancer.

2. KEYWORDS:

Magnetic Resonance Imaging, Prostate Cancer, Aggressiveness, Cancer Characterization, Metabolism, Perfusion, Non-Invasive, Hyperpolarized Carbon-13, Mutation Detection, Quantitative Imaging, Pre-clinical.

3. OVERALL PROJECT SUMMARY:

The initiation and first year of this project have gone extremely well accomplishing the milestones described in the original approved Statement of Work (SOW). No changes in the SOW were needed (or done). In this first year, the technical developments were accomplished and transgenic mouse model studies were initiated (as planned). Below we list each original specific aim from the SOW and describe the progress on each. Figures of the initial developments and data acquired are included. No publications have come out of this project yet, but we are on track to complete and submit manuscripts on both the technical developments and the results in the preclinical models in the second year.

Specific Aim 1. *ACQUISITION DEVELOPMENT FOR RAPID 3D HYPERPOLARIZED PERFUSION & METABOLIC MRI*

1a. Development of New Three-Resonance Excitation Pulses (months 1 – 2)

1b. Development of 3D volumetric Carbon-13 MRI sequence (months 1-6)

- Create and simulate response of new multi-band RF pulse designs for simultaneous ^{13}C-pyruvate, ^{13}C-lactate and ^{13}C-urea excitation
- Implement 3D volumetric echo planar sampling
- Test new acquisition method with in vitro solutions of ^{13}C-pyruvate, ^{13}C-lactate and ^{13}C-urea
- Optimize methods for highest spatial resolution, temporal resolution and SNR

Progress: A 3D dynamic MRSI sequence was designed to capture the metabolic and perfusion patterns. Spectral-spatial RF excitation was followed by double spin-echo refocusing and EPSI readout with random blips. (spectral BW= 581Hz, spectral res= 9.83Hz, FOV=4x4x8.6cm^3, res= 3.3x3.3x5.4mm^3) Multiband variable-flip excitation was applied to efficiently utilize magnetization[3,4]. To start, pyruvate is excited with

smaller flip angles than lactate and alanine, which effectively preserves pyruvate hyperpolarization to maintain high lactate and alanine SNR throughout the entire acquisition. A progressively increasing flip angle compensates for loss of magnetization due to each excitation pulse.

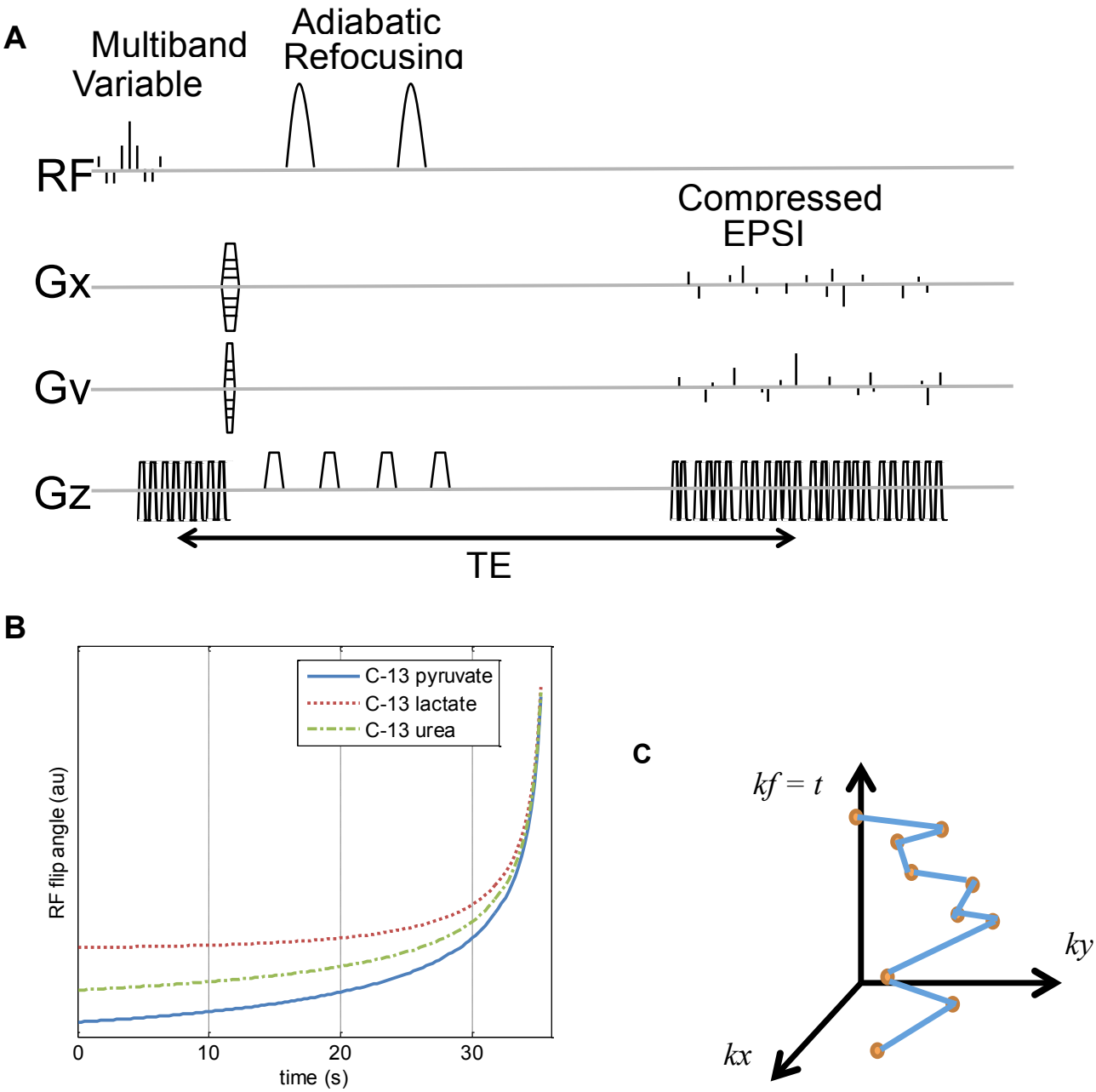


Figure 1. 3D dynamic pulse sequence designed for preclinical prostate cancer imaging with high spatiotemporal resolution A. The RF pulse features multiband excitation, variable flip angles, adiabatic double spin-echo refocusing and compressed sensing EPSI readout. B. Progressively increasing flip angles compensates for past RF excitations and T_1 relaxation. C. Compressed sensing using random blip in in-plane (x and y) gradients enables rapid acquisition of spectral-spatial images.

Specific Aim 2. *HP METABOLIC & PERFUSION IMAGING IN A TRANSGENIC MODEL OF PROSTATE CANCER WITH TISSUE ASSAY CORRELATIONS*

2a. Perform Perfusion & Metabolic Molecular Imaging with HP ^{13}C-pyruvate and HP ^{13}C-urea in 20 Early Stage Cancers (months 6 – 20)

2b. Perform Perfusion & Metabolic Molecular Imaging with HP ^{13}C-pyruvate and HP ^{13}C-urea in 20 Late Stage Cancers (months 6 – 20)

2c. Tissue assay determinations of cancer aggressiveness including histologic analysis, Ki-67 proliferative assays, lactate dehydrogenase (LDH) activity, *LDH-A* expression, cellularity, and micro-vessel density and hypoxia measurements (months 6 – 20)

- Imaging exams including the HP metabolic and perfusion MR techniques developed in Specific Aim 1.
- Subsequent pathologic, immunohistochemical analysis, and molecular assays. H&E staining as well as immunohistochemical markers for proliferation (KI-67) and micro-vessel density (CD31), hypoxia (pimonidazole, PIM) will be evaluated microscopically, mRNA will be isolated for RT-PCR analysis of LDH-A expression and LDH activity assays will be performed.
- Perform correlations of HP MR molecular imaging parameters with tissue analyses of cancer aggressiveness in 20 transgenic mice with low-grade early stage prostate cancers and 20 transgenic mice with high-grade cancers.

Progress: Following the technical development phase, we have initiated the preclinical studies in the transgenic model of prostate cancer studying both Early Stage and Late Stage cancers. Over the past 6 months we have tested and optimized the techniques *in vivo* and to date have studied a total of nine mice. Sample results are shown below and now that we have the techniques working well, we are confident that we can complete the proposed number of studies by the end of the project.

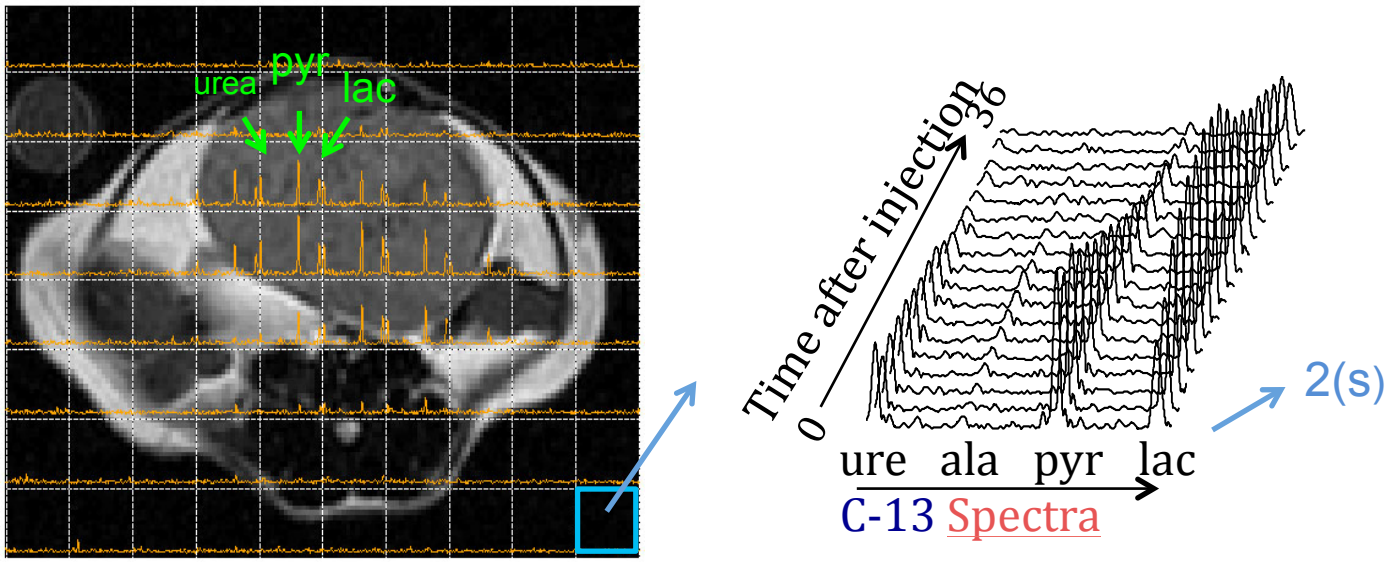
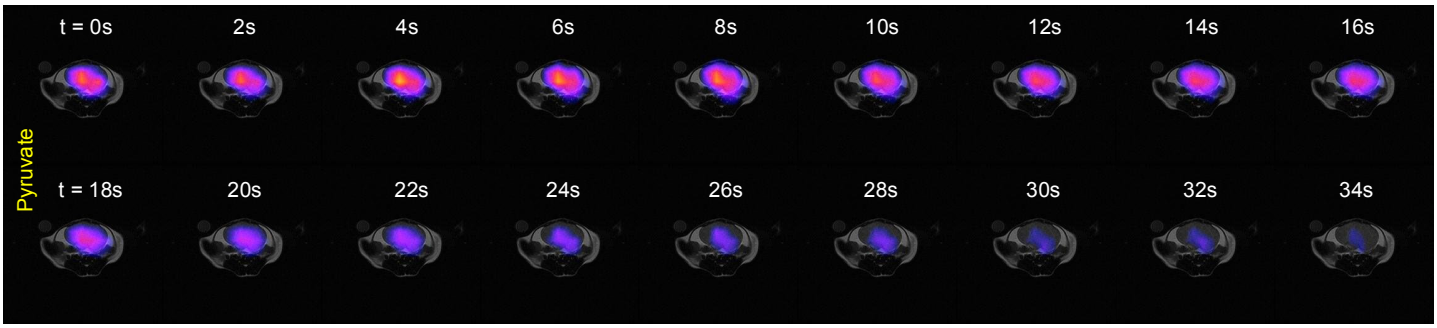


Figure 2. ***In vivo*** **MR spectroscopic imaging using the new 3D dynamic sequence**
Left: In this transgenic mouse with prostate cancer, concentration of ^{13}C tracers in different regions of the tumor mass can be visualized by 3.3mm x 3.3mm in-plane and 5.4mm resolution and voxel size of 0.058cm^3. The chemical shift spectrum is designed to efficiently use bandwidth and allows for sparsity in spectral direction. Note that aliasing are introduced to urea and lactate peaks. **Right:** The time-series spectra provides 2 second temporal resolution and optimal SNR at each time point for quantitative modeling of metabolism and perfusion/permeability.



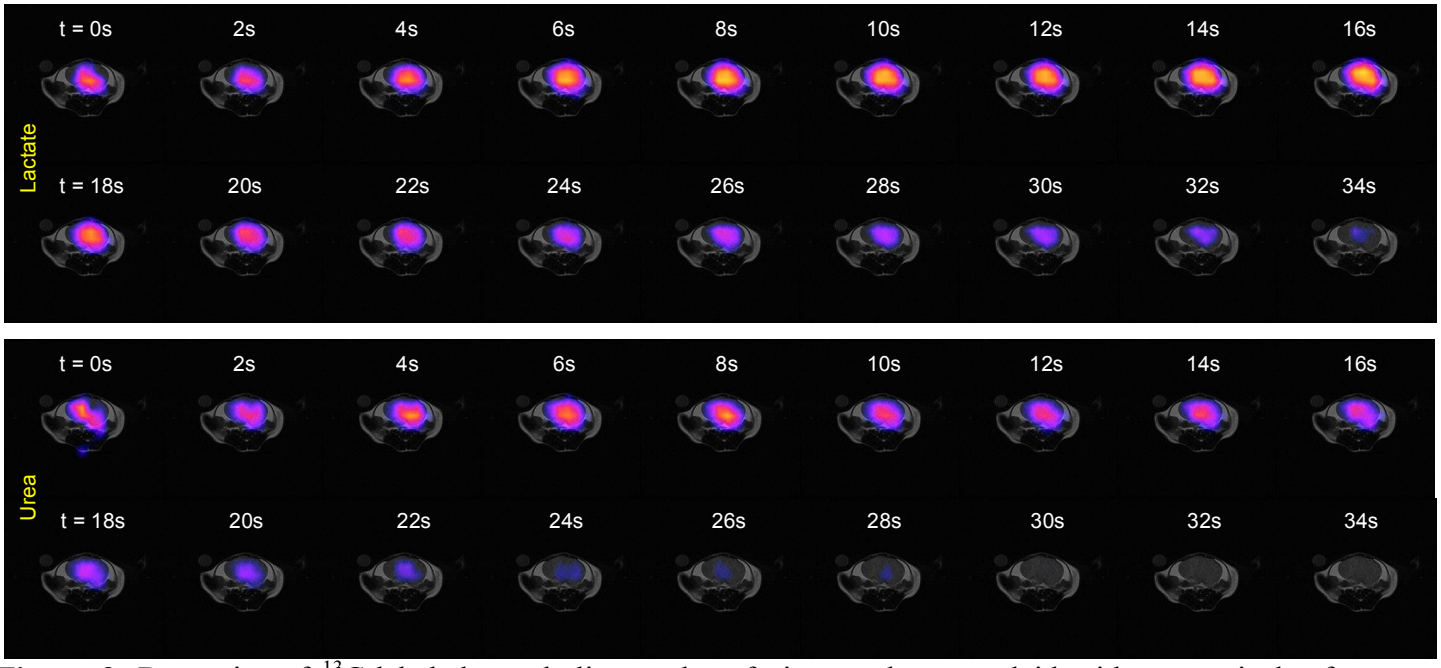


Figure 3. Dynamics of ^{13}C-labeled metabolites and perfusion markers overlaid with anatomical references. Shown are the time-series overlay of pyruvate (top), lactate (middle) and urea (bottom) starting from t = 0 after injection. The latency between pyruvate and lactate maximum in time shows their conversion in prostate tumor. Urea dynamics, on the other hand, illustrates the bolus arrival and perfusion into tumor regions.

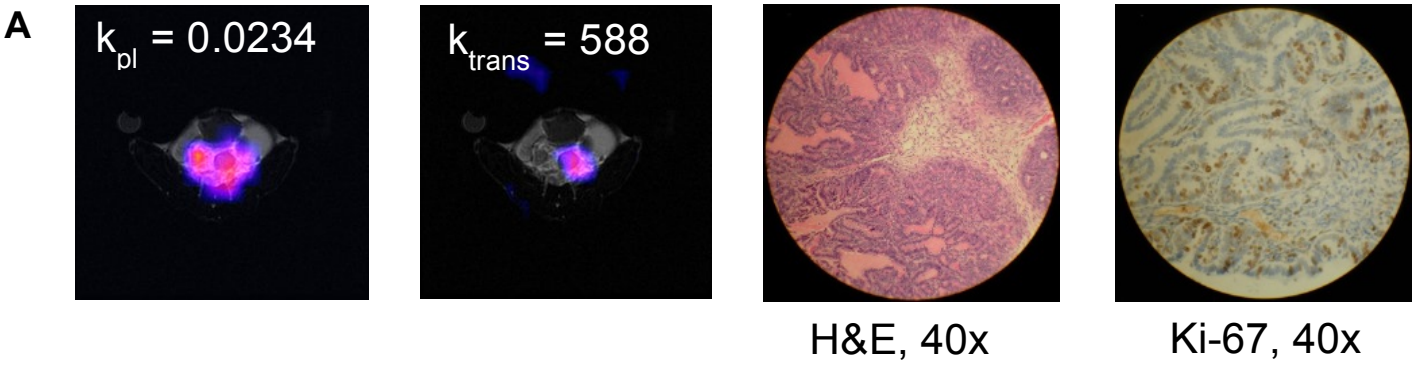
Metabolic and Perfusion Modeling: We implemented and tested specialized analysis methods to extract quantitative measures out of the dynamic hyperpolarized ^{13}C-pyruvate and ^{13}C-urea data. Tumor tissue exhibits elevated conversion from pyruvate to lactate. Conversion between pyruvate and lactate was modeled as:

$$\frac{dC_{lac}(t)}{dt} = k_{pl}C_{pyr}(t) - k_{lp}C_{lac}(t)$$

Tumor microcirculation can be characterized by the perfusion and permeability between blood and tissue (9). The perfusion dynamics can be described as (10):

$$\frac{dC_{tissue}(t)}{dt} = k_{trans}C_{blood}(t) - k_{ep}C_{tissue}(t)$$

Nonlinear fitting was applied to data for both models, where a correction was incorporated to account for the multiband excitation and variable flip angles. T_1 relaxation was assumed to be equal for all ^{13}C tracers. We have applied these methods in the initial preclinical model experiments and are currently continuing to test and optimize these techniques.



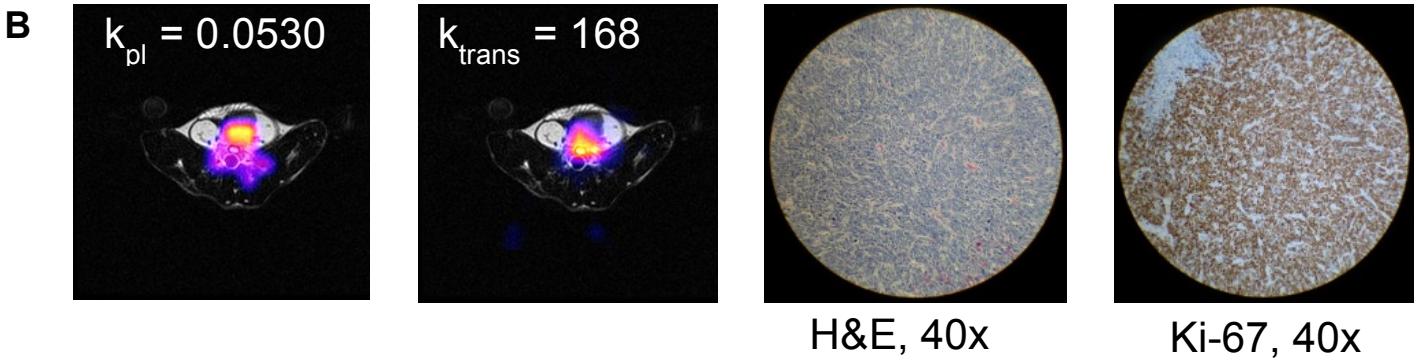


Figure 4. Quantitative metrics of metabolism and perfusion in two TRAMPs (**A** and **B**). The k_{pl} and k_{trans} maps, compared to histopathological analysis, suggest that metabolism and perfusion/permeability reflects fundamental pathophysiology in TRAMP mouse model of prostate cancer. In general, TRAMP prostate tumor shows elevated metabolism and altered perfusion/permeability versus normal tissue. Also, in the tumor (B) that histology indicated was more aggressive on H&E and Ki-67, the conversion rate from pyruvate to lactate (Kpl) was 2+-fold higher and perfusion measured by ktrans was 3.5-fold lower.

These initial findings support our hypotheses that aggressive prostate cancer would show higher metabolic flux through LDH to lactate and that adaptive changes in perfusion of the tumor microenvironment and increased cellularity would result in decreased perfusion in high-grade prostate cancers. In the next year, we will conduct the rest of the proposed studies and then be able to test these hypotheses in larger, more statistically significant numbers.

Specific Aim 3. *PERFORM HP METABOLIC/PERFUSION MRI EXPERIMENTS TO INVESTIGATE METASTATIC PROSTATE CANCER*

2a. Perform Perfusion & Metabolic Molecular Imaging with HP ^{13}C-pyruvate and HP ^{13}C-urea in 20 Transgenic Mice with Metastatic Prostate Cancers (months 12 – 24)

2a. Perform Tissue Sample Collection from both metastases and primary tumors following MR exam. (months 12 – 24)

2c. Tissue assay determinations of cancer aggressiveness including histologic analysis, Ki-67 proliferative assays, lactate dehydrogenase (LDH) activity, *LDH-A* expression, cellularity, and micro-vessel density and hypoxia measurements (months 12 – 24)

Progress: Following the study design, we have not yet performed the metastatic prostate cancer studies, but will in the next 12 months as planned.

4. KEY RESEARCH ACCOMPLISHMENTS:

- Developed new multiband RF pulses to simultaneously excite hyperpolarized ^{13}C-pyruvate, ^{13}C-lactate and ^{13}C-urea.
- Developed a novel volumetric acquisition sequence for obtaining hyperpolarized MR metabolic and perfusion images simultaneously.
- Created and tested specialized modeling techniques to provide quantitative measures of dynamic metabolic and perfusion data.
- Applied these new methods for the first time in preclinical models of Early-Stage and Late-Stage prostate cancers.

5. CONCLUSION:

This project has been designed to address the PCRP Overarching Challenges especially to "Distinguish aggressive from indolent disease". The goal of this project is to develop a new approach for simultaneous MR metabolic and perfusion imaging to distinguish aggressive prostate cancers from indolent disease based on up-regulated lactate-dehydrogenase (LDH) conversion of HP-pyruvate to lactate and altered vascular perfusion measured using HP-urea. We are obtaining the preclinical performance and safety experience needed for future FDA IND approval that is required before patient clinical trials can begin. These transgenic animal model

studies with correlations to pathologic and histochemical tissue assays of cancer aggressiveness provide preclinical accuracy data for the IND to justify the potential of this method to be effective (as well as safe) for future human studies. Also this method could ultimately provide new accurate assessments for monitoring patients on active surveillance, guiding biopsies to the sites of most abnormal perfusion/metabolism correlating with the most aggressive cancers, treatment selection and to monitor therapeutic response. Thus this new imaging approach could also have a role in addressing the other overarching challenge through the evaluation and iterative development of effective treatments for prostate cancer.

6. PUBLICATIONS, ABSTRCTS, AND PRESENTATIONS: "Nothing to report"

7. INVENTIONS, PATENTS AND LICENSES: "Nothing to report"

8. REPORTABLE OUTCOMES: "Nothing to report"

9. OTHER ACHIEVEMENTS: "Nothing to report"

10. REFERENCES:

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11. APPENDICES:

None.